SUMMARY OF THE INVENTION

Throughout this specification, unless the context requires otherwise, the word "comprise," or variations such as "comprises" or "comprising," will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

One aspect of the present invention contemplates an HBV variant exhibiting a replication fitness, in the presence of a nucleoside analogue, similar to or greater than in the absence of said nucleoside analogue.

Another aspect of the present invention provides an HBV variant carrying a mutation in the nucleoside sequence encoding a DNA polymerase resulting in an amino acid addition, substitution and/or deletion in said DNA polymerase in one or more amino acids as set forth in Formulae I and/or II:

FORMULA I

L, B_1, B_2, D, W, G, P, C, B_3, B_4, H, G, B_5, H, B_6, I, R, B_7, P, R, T, P, B_8, R, V, B_9, G, G, V, F, L, V, D, K, N, P, H, N, T, B_{10}, E, S, B_{11}, L, B_{12}, V, D, F, S, Q, F, S, R, G, B_{13}, B_{14}, B_{15}, V, S, W, P, K, F, A, V, P, N, L, B_{16}, S, L, T, N, L, L, S* (SEQ ID NO:1)

wherein:

B_1 is L, or R, or I

B_2 is E, or D

B_3 is T, or D, or A, or N, or Y

B_4 is E, or D

B_5 is E, or K, or Q

B_6 is H, or R, or N,

B_7 is I, or T

B_8 is A, or S

B_9 is T or R

B_{10} is A, or T, or S

B_{11} is R, or T

B_{12} is V, or G

B_{13} is S, or I, or T, or N, or V

B_{14} is T, or S, or H, or Y

B_{15} is R, or H, or K, or Q

B_{16} is Q, or P;

and

FORMULA II

S Z_1 L S W L S L D V S A A F Y H Z_2 P L H P A A M P H L L Z_3 G S S G L Z_4 R Y V A R L S S Z_5 S Z_6 Z_7 X N Z_8 Q Z_9 Z_{10} X X X Z_{11} L H Z_{12} Z_{13} C S R Z_{14} L Y V S L Z_{15} L L Y Z_{16} T Z_{17} G Z_{18} K L H L Z_{19} Z_{20} H P I Z_{21} L G F R K Z_{22} P M G Z_{23} G L S P F L L A Q F T S A I Z_{24} Z_{25} Z_{26} Z_{27} Z_{28} R A F Z_{29} H C Z_{30} Z_{31} F Z_{32} Y M* D D Z_{33} V L G A Z_{34} Z_{35} Z_{36} Z_{37} H Z_{38} E Z_{39} L Z_{40} Z_{41} Z_{42} Z_{43} Z_{44} Z_{45} Z_{46} L L Z_{47} Z_{48} G I H L N P Z_{49} K T K R W G Y S L N F M G Y Z_{50} I G (SEQ ID NO:2)

wherein:

X is any amino acid;

Z_1 is N or D;

Z_2 is I or P;

Z_3 is I or V;

Z_4 is S or D;

Z_5 is T or N;

Z_6 is R or N;

Z_7 is N or I;

Z_8 is N or Y or H;

Z_9 is H or Y;

Z_{10} is G or R;

Z_{11} is D or N;

Z_{12} is D or N;

Z_{13} is S or Y;

Z_{14} is N or Q;

Z_{15} is L or M;

Z_{16} is K or Q;

Z_{17} is Y or F;

Z_{18} is R or W;

Z_{19} is Y or L;

Z_{20} is S or A;

Z_{21} is I or V;

Z_{22} is I or L;

Z_{23} is V or G;

Z_{24} is C or L;

Z_{25} is A or S;

Z_{26} is V or M;

Z_{27} is V or T;

Z_{28} is R or C;

Z_{29} is F or P;

Z_{30} is L or V;

Z_{31} is A or V;

Z_{32} is S or A;

Z_{33} is V or L or M;

Z_{34} is K or R;

Z_{35} is S or T;

Z_{36} is V or G;

Z_{37} is Q or E;

Z_{38} is L or S or R;

Z_{39} is S or F;

Z_{40} is F or Y;

Z_{41} is T or A;

Z_{42} is A or S;

Z_{43} is V or I;

Z_{44} is T or C;

Z_{45} is N or S;

Z_{46} is F or V;

Z_{47} is S or D;

Z_{48} is L or V;

Z_{49} is N or Q;

Z_{50} is V or I; and

M^* is amino acid 550

and wherein S* in Formula I is designated as amino acid 420 and the first S in Formula II is designated as amino acid 421;

and wherein said variant exhibits a replication fitness in the presence of a nucleoside analogue similar to or greater than in the absence of said nucleoside analogue.

Yet another aspect of the present invention is directed to an HBV variant comprising a mutation in the nucleotide sequence encoding the HBV surface antigen which results in an amino acid addition, substitution and/or deletion in said surface antigen in a region corresponding to the amino acid sequences set forth in Formulae I and/or II wherein said variant exhibits a replication fitness in the presence of a nucleoside analogue similar to or greater than in the absence of said nucleoside analogue.

Even yet another aspect of the present invention is directed to an HBV variant comprising a mutation in the nucleotide sequence encoding the HBV surface antigen which results in an amino acid addition, substitution and/or deletion in said surface antigen in a region corresponding to the amino acid sequences set forth in Formulae I and/or II wherein said variant results in HBV DNA levels in the presence of a nucleoside analogue similar to or greater than the levels detected in pretreated patients.

Still yet another aspect of the present invention provides an HBV comprising a mutation in the nucleotide sequences encoding a DNA polymerase and a mutation in the nucleotide sequences encoding the surface antigen wherein each mutation results in an amino acid addition, substitution and/or deletion to each of the DNA polymerase and surface antigen and wherein said variants exhibits a replication fitness in the presence of a nucleoside analogue similar to or greater than in the absence of said nucleoside analogue.

Another aspect of the present invention contemplates a method for determining whether an HBV strain exhibits reduced sensitivity to a nucleoside analogue, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a

mutation in the nucleotide sequence encoding the DNA polymerase and optionally the surface antigen (listed below in parenthesis) wherein the presence of a T474N (P120T), M550V (I195M), M550I (W196S), L526M, W499S/W499Q (G145R) mutation, or combinations thereof or an equivalent one or more other mutation is indicative of a variant wherein said variant exhibits a replication fitness in the presence of a nucleoside analogue similar to or greater than in the absence of said nucleoside analogue.

Another aspect of the present invention contemplates a method for detecting an HBV agent which exhibits inhibitory activity to an HBV, said method comprising:

generating a genetic construct comprising a replication competent-effective amount of the genome from said HBV contained in a plasmid vector and then transfecting said cells with said construct;

contacting said cells, before, during and/or after transfection, with the agent to be tested;

culturing said cells for a time and under conditions sufficient for the HBV to replicate, express genetic sequences and/or assemble and/or release virus or virus-like particles if resistant to said agent; and

subjecting the cells, cell lysates or culture supernatant fluid to viral- or viral-component-detection means to determine whether or not the virus has replicated, expressed genetic material and/or assembled and/or been released in the presence of said agent.

Still another aspect of the present invention provides a method for detecting an HBV agent which exhibits inhibitory activity to an HBV, said method comprising:

generating a genetic construct comprising a replication competent-effective amount of the genome from said HBV contained in or fused to an amount of a baculovirus genome effective to infect cells and then infecting said cells with said construct;

contacting said cells, before, during and/or after infection, with the agent to be tested;

culturing said cells for a time and under conditions sufficient for the HBV to replicate, express genetic sequences and/or assemble and/or release virus or virus-like particles if resistant to said agent; and

FIG. 10 is a diagrammatic representation of a cross-section of a magnetic storage medium.

FIG. 11 is a diagrammatic representation of a cross-section of an optically readable data storage system.

ABBREVIATIONS

ABBREVIATION	DESCRIPTION
LAM	lamivudine
3TC	(LAM); (-)-β-2'-deoxy-3'-thiacytidine
HBIG	Hepatitis B immunoglobulin
HBV	Hepatitis B virus
ER	Endoplasmic reticulum

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

Lamivudine (LAM or 3TC) is a potent inhibitor of HBV replication. It is observed that HBV DNA titres are reduced in the sera of chronically infected patients after OLT and treatment with LAM and HBIG. LAM inhibits viral DNA synthesis. However, after a few months, there is an increase in HBV titres. Levels rose to pre-treatment levels. In accordance with the present invention, the inventors sequenced the genomes of the HBV resistant variants and revealed a number of mutations in the HBV polymerase gene which resulted in a level of replication fitness in the presence of a nucleoside analogue relative to its absence. Such a phenomenon is demonstratable by detecting viral load or burden in patients exposed to the nucleoside analogue. Viral load or burden is conveniently determined by detecting viral nucleic acid molecules (*e.g.*, DNA), replicative intermediates, polymerase activity, levels of surface antigen and/or titre of viral particles. The detection of such replication fit HBV variants in the presence of a nucleoside analogue is an important step in determining an appropriate therapeutic protocol for patients.

Accordingly, one aspect of the present invention contemplates an HBV variant exhibiting a replication fitness in the presence of a nucleoside analogue similar to or greater than in the absence of said nucleoside analogue.

Preferably, the HBV variant carries a mutation in the nucleotide sequence encoding the HBV DNA polymerase. Such mutation results in an addition, substitution and/or deletion of

an amino acid sequence of the DNA polymerase. Reference to the HBV DNA polymerase includes domains F and A through E set forth in Formula I below:

FORMULA I

L, B_1, B_2, D, W, G, P, C, B_3, B_4, H, G, B_5, H, B_6, I, R, B_7, P, R, T, P, B_8, R, V, B_9, G, G, V, F, L, V, D, K, N, P, H, N, T, B_{10}, E, S, B_{11}, L, B_{12}, V, D, F, S, Q, F, S, R, G, B_{13}, B_{14}, B_{15}, V, S, W, P, K, F, A, V, P, N, L, B_{16}, S, L, T, N, L, L, S* (SEQ ID NO:1)

wherein:

B_1 is L, or R, or I

B_2 is E, or D

B_3 is T, or D, or A, or N, or Y

B_4 is E, or D

B_5 is E, or K, or Q

B_6 is H, or R, or N,

B_7 is I, or T

B_8 is A, or S

B_9 is T or R

B_{10} is A, or T, or S

B_{11} is R, or T

B_{12} is V, or G

B_{13} is S, or I, or T, or N, or V

B_{14} is T, or S, or H, or Y

B_{15} is R, or H, or K, or Q

B_{16} is Q, or P;

and wherein S* is designated as amino acid 420.

In this specification, reference is particularly made to the conserved regions as defined by Poch *et al.* (16) as domains A to E (see also reference 17). Regions A to E are defined by the amino acid sequence set forth in Formula II below:

FORMULA II

S Z_1 L S W L S L D V S A A F Y H Z_2 P L H P A A M P H L L Z_3 G S S G L Z_4 R Y V A R L S S Z_5 S Z_6 Z_7 X N Z_8 Q Z_9 Z_{10} X X X Z_{11} L H Z_{12} Z_{13} C S R Z_{14} L Y V S L Z_{15} L L Y Z_{16} T Z_{17} G Z_{18} K L H L Z_{19} Z_{20} H P I Z_{21} L G F R K Z_{22} P M G Z_{23} G L S P F L L A Q F T S A I Z_{24} Z_{25} Z_{26} Z_{27} Z_{28} R A F Z_{29} H C Z_{30} Z_{31} F Z_{32} Y M* D D Z_{33} V L G A Z_{34} Z_{35} Z_{36} Z_{37} H Z_{38} E Z_{39} L Z_{40} Z_{41} Z_{42} Z_{43} Z_{44} Z_{45} Z_{46} L L Z_{47} Z_{48} G I H L N P Z_{49} K T K R W G Y S L N F M G Y Z_{50} I G (SEQ ID NO:2)

wherein:

X is any amino acid;

Z_1 is N or D;

Z_2 is I or P;

Z_3 is I or V;

Z_4 is S or D;

Z_5 is T or N;

Z_6 is R or N;

Z_7 is N or I;

Z_8 is N or Y or H;

Z_9 is H or Y;

Z_{10} is G or R;

Z_{11} is D or N;

Z_{12} is D or N;

Z_{13} is S or Y;

Z_{14} is N or Q;

Z_{15} is L or M;

Z_{16} is K or Q;

Z_{17} is Y or F;

Z_{18} is R or W;

Z_{19} is Y or L;

Z_{20} is S or A;

Z_{21} is I or V;

Z_{22} is I or L;

Z_{23} is V or G;

Z_{24} is C or L;

Z_{25} is A or S;

Z_{26} is V or M;

Z_{27} is V or T;

Z_{28} is R or C;

Z_{29} is F or P;

Z_{30} is L or V;

Z_{31} is A or V;

Z_{32} is S or A;

Z_{33} is V or L or M;

Z_{34} is K or R;

Z_{35} is S or T;

Z_{36} is V or G;

Z_{37} is Q or E;

Z_{38} is L or S or R;

Z_{39} is S or F;

Z_{40} is F or Y;

Z_{41} is T or A;

Z_{42} is A or S;

Z_{43} is V or I;

Z_{44} is T or C;

Z_{45} is N or S;

Z_{46} is F or V;

Z_{47} is S or D;

Z_{48} is L or V;

Z_{49} is N or Q;

Z_{50} is V or I; and

M^* is amino acid 550

and wherein the first S is designated as amino acid 421.

According, another aspect of the present invention provides an HBV variant carrying a mutation in the nucleoside sequence encoding a DNA polymerase resulting in an amino acid addition, substitution and/or deletion in said DNA polymerase in one or more amino acids as set forth in Formulae I and/or II:

FORMULA I

L, B_1, B_2, D, W, G, P, C, B_3, B_4, H, G, B_5, H, B_6, I, R, B_7, P, R, T, P, B_8, R, V, B_9, G, G, V, F, L, V, D, K, N, P, H, N, T, B_{10}, E, S, B_{11}, L, B_{12}, V, D, F, S, Q, F, S, R, G, B_{13}, B_{14}, B_{15}, V, S, W, P, K, F, A, V, P, N, L, B_{16}, S, L, T, N, L, L, S* (SEQ ID NO:1)

wherein:

B_1 is L, or R, or I

B_2 is E, or D

B_3 is T, or D, or A, or N, or Y

B_4 is E, or D

B_5 is E, or K, or Q

B_6 is H, or R, or N,

B_7 is I, or T

B_8 is A, or S

B_9 is T or R

B_{10} is A, or T, or S

B_{11} is R, or T

B_{12} is V, or G

B_{13} is S, or I, or T, or N, or V

B_{14} is T, or S, or H, or Y

B_{15} is R, or H, or K, or Q

B_{16} is Q, or P;

and

FORMULA II

S Z_1 L S W L S L D V S A A F Y H Z_2 P L H P A A M P H L L Z_3 G S S G L Z_4 R Y V A R L S S Z_5 S Z_6 Z_7 X N Z_8 Q Z_9 Z_{10} X X X Z_{11} L H Z_{12} Z_{13} C S R Z_{14} L Y V S L Z_{15} L L Y Z_{16} T Z_{17} G Z_{18} K L H L Z_{19} Z_{20} H P I Z_{21} L G F R K Z_{22} P M G Z_{23} G L S P F L L A Q F T S A I Z_{24} Z_{25} Z_{26} Z_{27} Z_{28} R A F Z_{29} H C Z_{30} Z_{31} F Z_{32} Y M* D D Z_{33} V L G A Z_{34} Z_{35} Z_{36} Z_{37} H Z_{38} E Z_{39} L Z_{40} Z_{41} Z_{42} Z_{43} Z_{44} Z_{45} Z_{46} L L Z_{47} Z_{48} G I H L N P Z_{49} K T K R W G Y S L N F M G Y Z_{50} I G (SEQ ID NO:2)

wherein:

X is any amino acid;
Z_1 is N or D;
Z_2 is I or P;
Z_3 is I or V;
Z_4 is S or D;
Z_5 is T or N;
Z_6 is R or N;
Z_7 is N or I;
Z_8 is N or Y or H;
Z_9 is H or Y;
Z_{10} is G or R;
Z_{11} is D or N;
Z_{12} is D or N;
Z_{13} is S or Y;
Z_{14} is N or Q;
Z_{15} is L or M;
Z_{16} is K or Q;
Z_{17} is Y or F;
Z_{18} is R or W;
Z_{19} is Y or L;

Z_{20} is S or A;

Z_{21} is I or V;

Z_{22} is I or L;

Z_{23} is V or G;

Z_{24} is C or L;

Z_{25} is A or S;

Z_{26} is V or M;

Z_{27} is V or T;

Z_{28} is R or C;

Z_{29} is F or P;

Z_{30} is L or V;

Z_{31} is A or V;

Z_{32} is S or A;

Z_{33} is V or L or M;

Z_{34} is K or R;

Z_{35} is S or T;

Z_{36} is V or G;

Z_{37} is Q or E;

Z_{38} is L or S or R;

Z_{39} is S or F;

Z_{40} is F or Y;

Z_{41} is T or A;

Z_{42} is A or S;

Z_{43} is V or I;

Z_{44} is T or C;

Z_{45} is N or S;

Z_{46} is F or V;

Z_{47} is S or D;

Z_{48} is L or V;

Z_{49} is N or Q;

Z_{50} is V or I; and

M^* is amino acid 550

and wherein S* in Formula I is designated as amino acid 420 and the first S in Formula II is designated as amino acid 421;

and wherein said variant exhibits a replication fitness in the presence of a nucleoside analogue similar to or greater than in the absence of said nucleoside analogue.

Preferred nucleoside analogues, including FAM and/or LAM and their chemical derivatives and homologues, are those which select mutations in the B and/or C domains of HBV polymerase.

Furthermore, in one particular embodiment, the nucleoside analogue selects a corresponding mutation in the HBV surface antigen gene resulting in an HBIG-resistant mutant. In another particularly preferred embodiment, the replication fitness HBV variant is selected following exposure to both the nucleoside analogue and HBIG treatment.

Accordingly, another aspect of the present invention is directed to an HBV variant comprising a mutation in the nucleotide sequence encoding the HBV surface antigen which results in an amino acid addition, substitution and/or deletion in said surface antigen in a region corresponding to the amino acid sequences set forth in Formulae I and/or II wherein said variants exhibits a replication fitness in the presence of a nucleoside analogue similar to or greater than in the absence of said nucleoside analogue.

In a related embodiment of the present invention, there is provided an HBV variant comprising a mutation in the nucleotide sequence encoding the HBV surface antigen which results in an amino acid addition, substitution and/or deletion in said surface antigen in a region corresponding to the amino acid sequences set forth in Formulae I and/or II wherein said variant results in HBV DNA levels in the presence of a nucleoside analogue similar to or greater than the levels detected in pretreated patients.

More particularly, the present invention provides an HBV comprising a mutation in the nucleotide sequences encoding a DNA polymerase and a mutation in the nucleotide sequences encoding the surface antigen wherein each mutation results in an amino acid addition, substitution and/or deletion to each of the DNA polymerase and surface antigen and wherein said variants exhibits a replication fitness in the presence of a nucleoside analogue similar to or greater than in the absence of said nucleoside analogue.